15 June 2017
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Innovax-ND-IBD (EMEA/V/C/004422/0000)

Common name: Newcastle disease, infectious bursal disease and Marek's disease vaccine (live recombinant)

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

On 1 September 2016 the applicant Intervet International B.V. submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Innovax-ND-IBD, through the centralised procedure falling within Article 3(1) of Regulation (EC) No 726/2004 (product developed by means of a biotechnological process).

The eligibility to the centralised procedure was agreed upon by the CVMP on 17 March 2016 as Innovax-ND-IBD has been developed by recombinant DNA technology.

The rapporteur appointed is Peter Hekman and the co-rapporteur is Esther Werner.

Innovax-ND-IBD is a live, genetically modified organism (GMO), frozen, cell-associated vaccine consisting of a recombinant herpes virus of turkeys (HVT, serotype 3, strain FC-126) genetically modified to contain the F gene from Newcastle disease virus (NDV) and the VP2 gene from infectious bursal disease virus (IBDV).

The vaccine is intended for active immunisation of one-day-old chicks by subcutaneous (SC) injection in order to reduce mortality and clinical signs caused by Newcastle disease (ND) virus, to prevent mortality and to reduce clinical signs and lesions of infectious bursal disease (IBD), and to reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus.

The product is a frozen cell suspension stored in liquid nitrogen. It is presented in 2 ml sealed glass ampoules containing 2 000 or 4 000 doses. The solvent is presented in plastic bags of 400 ml and 800 ml respectively. Dilution with the solvent for suspension for injection is required before injection into chicken.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 15 June 2017 the CVMP adopted an opinion and CVMP assessment report.

On 22 August 2017 the European Commission adopted a Commission Decision granting the marketing authorisation for Innovax-ND-IBD.

Scientific advice

Not applicable.

MUMS/Limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system has been provided (effective date: 15 October 2016) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, it is accepted that the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

The active substance and (frozen) vaccine are manufactured either at the Intervet Inc. site in Millsboro, in the US or at the Intervet International site in de Bilt, the Netherlands. These sites have a manufacturing authorisation issued on 27 January 2015 by the USDA and on 17 June 2014 by the ministry of Economic affairs of the Netherlands, respectively.

Batch release for the EU will be performed at the Intervet International site in Boxmeer, the Netherlands.

Batch release is performed at the Intervet International site in Boxmeer in the Netherlands.

For all the sites listed above, appropriate and valid Good Manufacturing Practice (GMP) certificates, which confirm the date of the last inspection and show that the site is authorised for the manufacture and batch release of such veterinary dosage forms, were presented.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit of the manufacturing site responsible for batch release.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance was considered in line with legal requirements.

The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily established, and is in line with the legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

The vaccine consists of a deep frozen suspension of cell-associated recombinant HVT containing the F gene from NDV and the VP2 gene from IBDV at a titre between $10^{3.3}$ and $10^{4.6}$ plaque forming units (PFU) per dose. Stabilizers (bovine serum, veggie medium) and a cryoprotectant (DMSO, dimethyl sulfoxide) are included in the formulation.

The solvent is a sterile, watery solution which contains stabilizers (sucrose, NZ-amine), a buffering agent (potassium dihydrogen phosphate) and a colouring agent (phenol red). The vaccine is mixed with the solvent prior to SC injection into chickens.

Container and closure

The vaccine is filled in 2 ml heat-sealed type I glass ampoules in accordance with European Pharmacopoeia (Ph. Eur. 3.2.1) requirements. The solvent is filled in 400 and 800 ml polyethylene (PE) (in accordance with Ph. Eur. 3.1.4 and 3.1.3 requirements) or multilayer plastic (MLP) (in accordance with Ph. Eur. 3.2.2.1 requirements) bags. Forming, filling, closing/sealing and terminal heat sterilisation of both types of bags is performed in a continuous process. Specifications and certificates demonstrating Ph. Eur. compliance were provided for the ampoules and bags.

Product development

Vaccines against MD, ND and IBD are routinely applied in poultry, the Innovax-ND-IBD recombinant HVT strain containing ND and IBD genes allows for a single SC vaccination of day-old chicks against these three important diseases. The vector, HVT, hardly spreads and is apathogenic to all avian species and not infectious to any other species. MD vaccines are administered to chickens at or before hatching because early immunity is essential. ND vaccination can be performed with live or inactivated NDV, this usually protects birds from serious consequences of disease but shedding may still occur. Because live vaccines may cause respiratory reactions, mild strains are normally used for primary vaccination. Maternally derived immunity may interfere with successful (live) vaccination and multiple applications are usually needed. Vaccination against IBDV is usually performed with live virus vaccines, MDA interferes with the development of immunity and the timing of vaccination therefore depends on the level of MDA in the flock. The use of a vector vaccine circumvents problems encountered with MDA for live vaccines against NDV and IBDV. The combined vector vaccine allows for a single vaccination against three important diseases requiring early onset of protection.

The vaccine strain is a HVT insertion mutant; the F gene of NDV and the VP2 gene of IBDV and regulatory sequences (promoters and polyA/terminator) have been inserted. Construction of the HVP360 was performed using standard techniques. Finally, the HVP360 virus was plaque purified.

The method of manufacture, formulation and storage is the same as for other live Marek vaccines routinely manufactured by the applicant.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC.

Description of the manufacturing method

Primary chicken embryo fibroblast (CEF) cell suspensions in veggie medium are prepared from SPF eggs using papain. The HVP360 virus seed is passaged on CEF monolayers to manufacture finished product. Cells are harvested using papain, centrifuged, counted, cell concentration is adjusted and stabiliser is added. The cell suspension is then filled in sterile 2 ml glass ampoules by automated filling and flame sealing. After labelling the product is frozen in a program freezer and stored in liquid nitrogen.

The solvent used is routinely supplied with existing MD vaccines from the applicant. The solvent is prepared by a simple mixing process and sterile filtered into a sterile bulk vessel. After interim storage the solution is filled into MLP or PE bags, followed by autoclaving.

The manufacturing of the active substance and the finished product are described in sufficient detail.

Production and control of starting materials

Active substance

The active substance, cell associated HVP360 virus, is manufactured and subsequently formulated and deep frozen. The master seed virus (MSV) was produced in CEF cells, supplemented with bovine serum and DMSO, filled in ampoules and stored in liquid nitrogen. Identity, sterility, absence of mycoplasma and absence of extraneous agents was tested on the master seed lot. Identity was performed by sequence analysis. Sterility, absence of mycoplasma and extraneous agents was tested in accordance with relevant Ph. Eur. monographs (resp. 2.6.1, 2.6.7 and 2.6.24).

Working seed virus (WSV) is prepared as described for the finished product. In the production facilities in the US, WSV is tested for sterility, absence of mycoplasma and extraneous agents in accordance with the Code of Federal Regulations Title (9 CFR) requirements, while in the EU facilities this is done in accordance with relevant Ph. Eur. monographs (resp. 2.6.1, 2.6.7 and 2.6.24).

Production and control of MSV and WSV are described in sufficient detail.

Excipients

Specifications of excipients and other starting materials (e.g. materials of biological and non-biological origin, media) are defined and analytical methods are provided.

Starting materials listed in a pharmacopoeia include: bovine serum (Ph. Eur. 2262), dimethyl sulfoxide (Ph. Eur. 0763), SPF chicken eggs (Ph. Eur. 5.2.2), sucrose (Ph. Eur. 0204), pancreatic digest of casein (UPS/NF), potassium dihydrogen phosphate (Ph. Eur. 0920), sodium hydroxide (Ph. Eur. 0677), hydrochloric acid (Ph. Eur. 0002), phenolsulfonphthalein (Ph. Eur. 0242) and water for injections (Ph. Eur. 0169).

Starting materials not listed in pharmacopoeia include: veggie medium, veggie protease (papain) and leupeptin; example certificates of analysis are provided.

Information on the use of antibiotics and possible traces of antibiotics in the final product were provided. Information with regard to the suppliers of SPF eggs was provided.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies (TSE)

The documentation provided for all the materials of animal origin (HVP360 seed virus, SPF chicken eggs and bovine serum and casein) demonstrated their compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) and Commission Directive 1999/104/EEC.

Moreover, the product is intended for poultry, a non TSE susceptible species.

It is concluded that, the risk of transmitting TSE infectivity through the use of this vaccine is negligible.

Control tests during the manufacturing process

During manufacture the following in-process control tests are carried out to ensure the quality parameters: check on cytopathic effect (CPE), cell count (determination of viable cells), check on filling volume.

A check on cytopathic effect is performed by microscopic observation, typical CPE must be observed. A cell count is performed, by microscopy or equivalent system and should meet the specification. The filling volume is checked at regular intervals during filling. Test descriptions and limits of acceptance are presented.

The in-process controls are appropriate, adequately performed and described. The results of in-process controls of three consecutive production runs of the vaccine are provided. All three batches were within the acceptance limits for the three in-process control tests. In-process control tests are considered in line with the requirement of current guidelines.

No in-process control tests during production of the solvent are performed.

Control tests on the finished product

General tests (e.g. appearance) are not performed on the finished product, since it is deep frozen.

Identity of active substance and potency tests are combined in one virus titration performed on each batch of final product. Virus titre is calculated. The release requirement for a batch is at least $10^{3.3}$ PFU/dose but not more than $10^{4.6}$ PFU/dose. Separate assays are performed, confirming the identity of the virus and the presence of both inserted genes. Appropriate validation of the test was performed.

A sterility test is performed in accordance with Ph. Eur. 2.6.1 and 0062 requirements. Two methods may be used for routine testing. The suitability of the sterility test methods was appropriately validated.

A test for absence of mycoplasmas is performed in accordance with Ph. Eur. 2.6.7. Two methods can be used for routine testing. The suitability of the methods for testing the absence of mycoplasma was appropriately validated.

A test for absence of extraneous agents is performed in accordance with Ph. Eur. 2.6.25 requirements.

General tests are performed on the solvent: pH value (in accordance with Ph. Eur. 2.2.3 requirements), sucrose and potassium phosphate content (in accordance with Ph. Eur. 2.3.1), clarity (in accordance with Ph. Eur. 2.2.1 requirements), appearance (colour) and filling volume. Appropriate limits have been set for each of these tests. The HPLC test for sucrose was appropriately validated. Identity of the solvent is confirmed by the presence of sucrose, phenolsulfonphthalein, potassium and phosphate, as determined by the general tests. The sterility of the solvent is tested in accordance with Ph. Eur. 2.6.1. requirements.

Batch-to-batch consistency

Results of finished product tests for 3 consecutive batches are provided; all batches conform to the release requirements. Results of finished product tests for 3 consecutive batches of the solvent for each of the two packaging materials (PE bags and MLP bags) are presented and conform to the release requirements.

Stability

The finished product is stored in liquid nitrogen for a maximum of 24 months. In order to support the proposed shelf life, the applicant has initiated a 39 month stability study. The product was tested for HVP360 titre (PFU/vial) only, since tests for integrity of the closure are not required in airtight sealed glass ampoules. Results up to 27 months (3 pilot batches) and 18 months (3 commercial batches) show no decrease of titre. Considering the available data and the known stability of this type of vaccine a shelf life of 24 months is considered supported.

In-use stability was investigated by dissolving one ampoule each of three batches of finished product in 200 ml of solvent. Samples were taken after dilution and at 1, 2 and 3 hours of storage at 15-25°C. Results show a slight decrease in titre for two out of three batches over the three hours storage. A two hour in-use period is justified from the data provided.

A stability study was performed using different presentations of diluent. Sucrose, pH, clarity, appearance and sterility were tested at regular intervals. Bags were stored at 25°C for a maximum of 36 to 45 months (PE bags) or 24 months (MLP bags). All parameters remained within specifications throughout the storage periods. The shelf life of 36 months for the diluent in PE bags and 24 months for diluent in MLP bags is considered justified.

Overall conclusions on quality

Innovax-ND-IBD is a live recombinant vaccine for active immunisation of chickens against MD, ND and IBD. The vaccine is available in ampoules containing 2000 or 4000 doses and is diluted before use in solvent supplied in plastic bags. Satisfactory information on the ampoules and bags was provided.

Information regarding the qualitative and quantitative composition is provided and is acceptable. One dose of vaccine contains ≥ $10^{3.3}$ and ≤ $10^{4.6}$ PFU of Innovax-ND-IBD virus strain HVP360 as active ingredient. The virus is grown on chicken embryo fibroblast (CEF) cells produced from embryos obtained from SPF chicken flocks. The manufacturing method can be considered as standard for this type of vaccine and is well described with sufficient details. Cells containing the virus are harvested and combined with serum and a cryoprotectant (DMSO) to allow storage in liquid nitrogen.

Starting materials listed in a pharmacopoeia are of satisfactory quality.

Appropriate procedures have been implemented to ensure the absence of extraneous agents in starting materials of animal origin. A TSE risk assessment was performed. The risk that the final product may transmit TSE to the target animal is negligible.

The production method, including in-process controls and quality control on the finished product together with control of the starting materials, ensure a consistent quality of batches of vaccine. The whole production process was satisfactorily evaluated at production scale.

Results of the stability tests for the final product showed no loss in infectivity titre during a 27 month storage period in liquid nitrogen. The data are considered to support the proposed 24 month shelf life. Stability data of reconstituted product show that the vaccine remains relatively stable at room temperature for 3 hours, therefore the proposed 2 hours in-use shelf life can be accepted.

In conclusion, the production process is adequately described and controls in place are appropriate to ensure the quality of the product at release and throughout the shelf life.

Part 3 – Safety

Introduction and general requirements

Studies to determine the safety of the vaccine were performed in accordance with the Ph. Eur. monographs 0062 on vaccines for veterinary use, Ph. Eur. chapter 5.2.6 on evaluation of safety of veterinary vaccines and immunosera, Ph. Eur. monograph 0589 on Marek's disease vaccine (live) and in accordance with the Commission Directive 2009/9/EC amending Directive 2001/82/EC and the regulations for GMOs.

Safety documentation

The safety of the vaccine was investigated in nine (9) laboratory and three field studies.

In the safety studies included in the initial application, Innovax-ND-IBD was administered to 1-day old chickens usually according to the recommended vaccination scheme by the SC route. In three of the studies the *in ovo* administration of the vaccine is included additionally (overdose) or alternatively (dissemination, reversion to virulence). The full data and claim for the *in ovo* administration route are expected to be added to the dossier later, via a variation procedure. Batches used in the safety studies contained maximum potency dose (by lowest passage level).

Laboratory tests

The following GLP compliant studies were carried out with an Innovax-ND-IBD batch representing the lowest passage level that may be produced in 1-day old SPF chickens, the minimum age recommended for vaccination and the most sensitive category of the target species:

The applicant has provided a study in which a tenfold overdose ($10^{5.6}$ PFU/dose) of Innovax-ND-IBD was administered SC (and *in ovo*). Further studies were carried out investigating the spread of an overdose of Innovax-ND-IBD from vaccinated chickens to contact chickens and contact turkeys as well as replication and spread in several non-target avian species (turkeys, ducks, pheasants, quails). The applicant also investigated the dissemination of the modified HVT vaccine strain HVP360 in chickens in comparison to its parental strain, and the possible reversion to virulence over five back passages.

Potential interactions between Innovax-ND-IBD and Nobilis ND Clone 30 or Nobilis ND C2 (both vaccines against Newcastle Disease) applied on the same day at different sites were investigated as well as potential interactions between Innovax-ND-IBD and Nobilis IB Ma5 mixed with Nobilis IB 4-91. In addition, three field studies were conducted examining the safety of 1-day old broiler chickens.

The safety of a single dose, the repeated administration of a single dose and safety for the reproductive tract were not investigated, but this was adequately justified by the applicant. Additionally two studies were included in this application: one on spread to one mammalian species (mice) and one examining the possibly altered immunological functions. Both studies were conducted with a similar product: Innovax-ILT.

A discussion of the results of the various studies is included under the relevant headings below.

Safety of the administration of one dose

The safety of the administration of one dose was not studied; this is in line with Ph. Eur. 0589, Marek's disease vaccine (live). The safety of administration of an overdose is considered to cover safety of a single dose, this is acceptable.

Safety of one administration of an overdose

The pivotal safety study was conducted in compliance with the principles of Good Laboratory Practice (GLP) and designed to meet both Ph. Eur. 0589 and 9 CFR requirements.

The safety of a tenfold overdose was tested in the following vaccination groups:

- Group 1: 70 chicken eggs were inoculated at day 18 ($10^{5.1}$ PFU/dose)
- Group 2: 50 chickens were vaccinated at 1-day old with 10x overdose ($10^{5.6}$ PFU/dose)
- Group 3: 70 chicken eggs remain as unvaccinated controls
- Group 4: 70 chickens eggs were inoculated at day 18 with solvent only
- Group 5: 50 chickens were inoculated with virulent MDV at the age of 8 days to demonstrate the susceptibility of the used SPF chickens.

In accordance with 9 CFR, the challenge control group was followed-up for 50 days instead of the 70 days described in the Ph. Eur. This is acceptable since the shorter observation period and the higher percentage of chicks that need to be MD positive for 9 CFR represent a stricter requirement to show the susceptibility of the chicks.

Hatchability was 91, 93, and 93% in groups 1, 3, and 4. For the observation period animal numbers were reduced to 50 and all groups were observed for 124 days. No clinical signs related to the vaccination were noted in any group besides group 5 which was challenged with virulent MDV on day 8. The test is considered valid in line with Ph. Eur. 0589 requirements. It can therefore be concluded that the vaccine can be safely applied via the SC route to day-old chicks, with a maximum titre of $10^{4.6}$ PFU/dose.

On the basis of the results no safety concerns arose following the administration of an overdose ten times higher than the recommended dose to the chicks.

Safety of the repeated administration of one dose

No study on safety of the repeated administration of one dose has been provided. Since vaccination consists of a single lifetime injection at one day of age, it is not required to study safety of the repeated administration of one dose.

Examination of reproductive performance

No specific studies on the safety for the reproductive tract were performed. This is considered acceptable as there are no data to suggest negative effects of the parent strain on the reproductive tract. Moreover, it is considered unlikely that the addition of the ND and IBD genes changed the safety profile of the parent strain. However, since no data have been provided concerning safety of HVP360 for the reproductive tract a warning sentence in the SPC is considered appropriate.

Examination of immunological functions

The parent strain HVT is apathogenic and it is not known to be immunosuppressive in chickens. A study was performed on the immune response after vaccination with Innovax-ILT, which is constructed using the same HVT backbone as Innovax-ND-IBD, with two ILT genes inserted.

The potential immunosuppressive properties of vaccine strain HVT/ILT-138 were investigated by a vaccination-challenge experiment through vaccination with Nobilis ND Clone 30 two weeks after vaccination with Innovax-ILT at 1-day of age.

One group of chickens was vaccinated with a tenfold overdose of Innovax-ILT and 14 days later with Nobilis ND Clone 30. This time point was probably chosen as in another study the peak of viraemia was observed at two weeks post vaccination with Innovax-ILT. For comparison the second group was only vaccinated with Nobilis ND Clone 30. An unvaccinated control group was included. Three weeks afterwards all groups were challenged with a velogenic NDV strain Herts 33/56 (required by Ph. Eur. Monograph 0450) and were clinically observed.

Both vaccination groups were fully protected against challenge. No lesions related to the challenge virus could be found after sacrifice. All unvaccinated control chickens were unprotected, rapidly developed severe clinical signs of ND and were euthanized.

The study using Innovax-ILT is considered to be relevant for Innovax-ND-IBD and provides sufficient assurance for the absence of negative effects on immunological functions.

Special requirements for live vaccines

Spread of the vaccine strain

Three studies were performed to investigate spread of the vaccine strain to target and non-target species as well as safety and spread of the vaccine strain in non-target species. The species studied included turkeys, pheasants, ducks and quail which are considered to be the relevant species. Studies were appropriately designed and of sufficient quality.

The potential spread of Innovax-ND-IBD to target animals (SPF chickens) and non-target animals (turkeys) was investigated with a tenfold overdose using the recommended route of administration (SC). The presence of the virus strain in vaccinated animals and sentinels was evaluated at 3 time points.

One group was composed of an equal number of vaccinated chickens and unvaccinated chickens as sentinels housed together (34 + 34 chickens). A second group was divided in vaccinated chickens housed together with the same number of unvaccinated turkeys (34 + 34 birds). Spread of the vaccine from chickens to in-contact chickens or turkeys was not shown.

The potential replication and spread of the vaccine strain HVP360 was evaluated in 4 potentially susceptible non-target species: turkeys, ducks, pheasants and quails (commercial birds). These are the potentially susceptible species most likely used for commercial purpose and housed in close proximity to the chicken sites.

The birds were vaccinated at 1 to 21 days of age, which should have no impact on the results. All birds were divided per species in a vaccinated sub-group and an unvaccinated sub-group using equal numbers of animals (each including 26 birds). The vaccine was given at a tenfold overdose. Birds were observed for 120 days except for the ducks which were observed only for 50 days as by this time no virus replication or spread could be detected. However, spread between turkeys was observed. None of the species tested showed any clinical signs or macroscopic lesions of HVT after vaccination with a tenfold overdose and therefore the vaccine is considered safe for non-target species. When the vaccine is used as directed, spread is unlikely to occur. The conclusion that the HVP360 host range has not changed compared to the parent strain is supported. An appropriate warning sentence that the vaccine strain is excreted from vaccinated birds and may spread to turkeys is included in the SPC.

A study was provided evaluating the safety and spreading of an overdose of Innovax-ILT subcutaneously administered to mice. From these data it can be concluded that Innovax-ILT is safe in mice and unable to replicate and spread. Based on the data provided and the properties of the parent strain, it is considered likely this holds true for other mammals and for Innovax-ND-IBD as well. The risk of Innovax-ND-IBD posing a threat to mammals is considered to be negligible.

On the basis of the above it is concluded that the vaccine virus can spread to in-contact unvaccinated animals. An appropriate warning sentence that the vaccine virus is excreted from vaccinated birds and may spread to turkeys is included in the SPC.

Dissemination in the vaccinated animal

The tissue tropism of the HVP360 virus strain was compared to that of the parent strain HVT FC-126 after *in ovo* vaccination of two groups of 50 chicken embryos. For this study Innovax-ND-IBD was used at comparable titres with $10^{5.1}$ PFU/dose and Nobilis Marexine CA126 with $10^{4.9}$ PFU/dose. Hatchability was at 86% resp. 88% compared to the unvaccinated control group with 93% which is acceptable. No clinical signs related to the vaccination were noted during the study.

The results show that the HVT FC-126 virus replicated slightly better in most tissues, which was most apparent in the feather follicles where HVP360 was almost absent which may indicate a decreased shedding and therefore a lower ability to spread. The study was appropriately designed and executed. Based on the data provided, the dissemination of the vaccine strain is not significantly different from the parent strain.

Reversion to virulence of attenuated vaccines

A reversion to virulence study was performed, in accordance with Ph. Eur. requirements. For this study the vaccine strain HVP360 was inoculated with $10^{5.1}$ PFU/dose in 30 18 days old SPF chicken eggs. Twenty two chicks hatched (hatchability 80%) and 15 of them were allocated in group 1. Five passages in 1-day old chicks were performed after preparation of white blood cell suspension for inoculation.

No effects on hatchability and no clinical signs or macroscopic lesions were observed at the last passage. It can be concluded that the vaccine virus did not acquire virulence during passaging.

Biological properties of the vaccine strain

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strain. The vaccine virus (HVP360) is based on the naturally apathogenic HVT FC-126 strain where the F gene of NDV and the VP2 gene of IBDV have been inserted into the genome. The HVP360 strain expresses NDV F and IBDV VP2 proteins. Based on the results of safety studies it is concluded that the well-known biological properties of the vaccine strain have not changed when compared to the parent HVT FC-126 strain.

Recombination or genomic reassortment of the strains

The probability of recombination or genomic reassortment with field or other strains was considered in line with the requirements of Directives 2001/82/EC and 2001/18/EC. The applicant has sufficiently addressed the biological properties of the vaccine strain and the risk of recombination or genomic reassortment occurring. The risk is considered to be negligible.

User safety

An assessment of risks associated with the use of the vaccine was performed in accordance with the CVMP guideline for user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006). No hazards were identified for the solvent. The user may be exposed to the vaccine by accidental self-injection, or when a glass ampoule explodes when thawed. The risk of exposure is considered to be moderate. The vaccine virus can however only infect avian species, while DMSO and other components are considered to be safe. The consequence of skin exposure or accidental self-injection or inhalation after explosion of an ampoule is therefore negligible. The overall risk of skin exposure and accidental injection is negligible, the inclusion of a warning sentence is therefore not considered necessary. The consequences of an ampoule exploding are estimated to be medium (skin cuts), the overall risk is therefore medium/low. In order to mitigate this risk (skin cuts), appropriate warnings and mitigation measures have been included in the SPC.

Study of residues

No specific studies were performed. This is considered appropriate.

MRLs

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product. Some constituents (bovine serum, veggie medium and phenolsulfonphtalein) are not included in table 1 of Regulation (EU) No 37/2010 or in the list of substances not falling within the scope of Regulation (EC) No 470/2009. They are considered not pharmacologically active at the dose used.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

Innovax-ND-IBD can be given on the same day but via different administration routes with the live Newcastle vaccines Nobilis ND Clone 30 or Nobilis ND C2 or the infectious bronchitis vaccines Nobilis IB Ma5 mixed with Nobilis IB 4-91. Two studies were performed to investigate the safety of the associated use of these vaccines.

First study: The safety of administration of Innovax-ND-IBD and Nobilis ND Clone 30 or Nobilis ND C2 was investigated in line with Ph. Eur. 0450 requirements regarding Newcastle disease vaccines (live). Fifteen SPF chickens in each group were vaccinated with Innovax-ND-IBD (SC) and at the same time with a dose of Nobilis ND C2 or Nobilis ND Clone 30 (ocular). Appropriate control groups were included. No clinical signs were observed in any of the groups during a 14-day observation period. It can be concluded it is safe to administer Innovax-ND-IBD on the same day as Nobilis ND C2 or Nobilis ND Clone 30.

Second study was performed to assess the safety of Innovax-ND-IBD given on the same day but via different administration routes with Nobilis IB Ma5 mixed with Nobilis IB 4-91, in line with Ph. Eur. 0442 (Avian infectious bronchitis vaccine (live)) requirements. SPF chickens (44 or 32 animal per group) were vaccinated with Innovax-ND-IBD (SC) either alone or concurrently with Nobilis IB Ma5 mixed with Nobilis IB 4-91 by oculo-nasal inoculation and appropriate control groups were included. No clinical signs were observed after vaccination, all groups passed the safety requirements of Ph. Eur. 0442. Histology kidney scores were low in all groups while there was no difference in average ciliostasis scores between the groups vaccinated with Nobilis IB Ma5 mixed with Nobilis IB 4-91 given alone or concurrently with Innovax-ND-IBD or Nobilis Marexine CA126 (contains the HVT parent strain).

Both studies on safety of concurrent use of Innovax-ND-IBD with other vaccines were appropriately designed and executed. It can be concluded that it is safe to use Innovax-ND-IBD at the same time as Nobilis ND C2 or Nobilis ND Clone 30 or at the same time as Nobilis IB Ma5 mixed with Nobilis IB 4-91. This is reflected in the SPC.

Field studies

Three controlled randomized combined safety and efficacy field studies were performed. Two semi-field studies in the UK in which containment was in place because of the nature of the vaccine (live GMO) and one standard field study in the Netherlands. Studies were performed in accordance with GCP.

The two semi-field studies were appropriately designed, and generally of sufficient quality. In the first study, 5555 1-day old chicks were vaccinated first with Nobilis IB Ma5 and subsequently with Innovax-ND-IBD or with the solvent alone. There were no obvious indications of safety problems. However, due to problems with increased mortality in vaccinates during the study (50%), most likely as a result of circumstances during transport, it cannot be accepted as fully supportive of the safety of the vaccine in the field.

In the second study, 4387 1-day old chicks were vaccinated first with Nobilis IB Ma5 and subsequently with Innovax-ND-IBD or with the solvent alone. An infection with *E. coli* caused higher mortality in both groups in the first part of the study. However, overall similar mortality rates were observed and the study generally supports the safety of the vaccine.

The third field study, performed in the Netherlands, results indicate no significant differences between vaccinated and control groups with respect to general health and daily mortality. The study generally supports the safety of the vaccine when used under field circumstances.

In conclusion, the vaccine was shown to be safe under field circumstances.

Environmental risk assessment

A Phase 1 Assessment of environmental risk was performed in accordance with the CVMP note for Guidance (EMEA/CVMP/074/95). Based on the data provided the environmental risk assessment can stop at Phase I. Innovax-ND-IBD is not expected to pose a risk for the environment when used according to the SPC.

Environmental risk assessment for products containing or consisting of genetically modified organisms

An environmental risk assessment in accordance with Directive 2001/18/EC is provided, including a copy of the written consent of the Dutch authorities for the deliberate release in the environment as this vaccine is a GMO.

The applicant has provided sufficient information on the origins, method of recombination, stability, biological properties and genomic sequence of the vaccine strain. Potential risks related to release of GMO in the environment were appropriately addressed, based on data from studies as well as literature. The conclusion is that the risk to both humans and the environment is negligible.

Overall conclusions on the safety documentation

The safety of Innovax-ND-IBD was investigated in nine laboratory studies.

The application of a tenfold overdose of Innovax-ND-IBD to chickens of the youngest age to be treated was found to be safe. Reproductive performance was not studied, but a warning is included in the SPC, which is considered acceptable. Specific studies regarding the influence of Innovax-ND-IBD on the immune system were not performed but appropriate justification was provided.

The vaccine strain is considered to be able to spread from vaccinated chickens via feather dust. The application of a tenfold dose of the vaccine to turkeys, pheasants, ducks and quails was shown to be safe. Innovax-ND-IBD is a GMO vaccine. The biological properties of Innovax-ND-IBD are comparable to its parental HVT strain (FC-126) in terms of chronology of virus appearance and tissue tropism. The risk of Innovax-ND-IBD posing a threat to mammals is considered to be negligible.

A reversion to virulence study was performed, it could be concluded the virus did not acquire virulence

during passaging. The applicant has sufficiently addressed the biological properties of the vaccine strain and the risk of recombination or genomic reassortment occurring is considered to be negligible.

Residue studies are not required. The withdrawal period is set at zero days.

The safety of the vaccine was also investigated in three field studies carried out in the UK and Netherlands in accordance with GCP. The vaccine was shown to be safe under field circumstances

The safety of the associated use (at the same day by different routes) of Innovax-ND-IBD with Nobilis ND C2 or Nobilis ND Clone 30 and of Innovax-ND-IBD with Nobilis IB Ma5 mixed with Nobilis IB 4-91 was demonstrated.

The user safety has been adequately addressed and appropriate warnings are included in the SPC.

Based on the data provided the ERA can stop at phase I. An environmental risk assessment in accordance with Directive 2001/18/EC is provided, including a copy of the written consent of the Dutch authorities for the deliberate release in the environment. The applicant has provided sufficient information on the origins, method of recombination, stability, biological properties and genomic sequence of the vaccine strain. Potential risks related to release of GMO in the environment were appropriately addressed, based on data from studies as well as literature. The conclusion that the risk to both humans and the environment is negligible can be supported.

The vaccine virus strain was shown to be genetically stable with no risk of reversion to virulence.

In conclusion, when used as directed, the vaccine is considered to be generally safe for the target animal, the environment, the user and the consumer.

Part 4 – Efficacy

General principles

Innovax-ND-IBD is intended for active immunisation of one-day-old-chicks by SC administration of a minimum dose of $10^{3.3}$ PFU.

The product is indicated to:
- reduce mortality and clinical signs caused by NDV
- prevent mortality and reduce clinical signs and lesions caused by IBDV
- reduce mortality, clinical signs and lesions caused by MDV

The proposed onset of immunity (OOI):	The proposed duration of immunity (DOI):
MD: 9 days	MD: entire risk period
ND: 4 weeks	ND: 8 weeks
IBD: 2 weeks	IBD: 8 weeks

The applicant states that Innovax-ND-IBD can be used on the same day (at different administration sites) with: Nobilis ND Clone 30 (OOI for ND component: 3 weeks) or Nobilis ND C2 (OOI for ND component: 2 weeks) or Nobilis IB Ma5 or Nobilis IB 4-91.

Introduction and general requirements

The vaccine strain HVP306 is an HVT vector vaccine, which expresses the NDV F gene and the IBDV VP2 gene. The F gene is derived from the ND vaccine strain Clone 30, the VP2 gene is derived from IBDV strain Faragher 52/70 which is a classical pathogenic strain. All three viruses are relevant for the

European situation.

The following monographs were used as a basis for the designs of the efficacy studies: Ph. Eur. 0589: Marek's disease vaccine (live), Ph. Eur. 0450: Newcastle disease vaccine (live) and Ph. Eur. 0587: Avian infectious bursal disease vaccine (live).

In total twenty-one (21) laboratory vaccination challenge studies and three field studies were performed to substantiate the efficacy claims made for this product. OOI and DOI studies in SPF chickens at the recommended age for vaccination (1 day) and the influence of maternal derived antibodies (MDA) was investigated for the three diseases against which the vaccine is intended to afford protection, in each case using vaccine batches at or below the minimum dose. In addition, the concurrent use with live ND and IB vaccines was investigated.

Primary efficacy parameters were chosen in accordance with respective Ph. Eur. monographs. For MD: the relative protection percentage is based on deaths, animals with clinical signs and macroscopic lesions of Marek's disease that should be no less than 80%. For ND: not fewer than 90% of chickens should survive and show no notable clinical signs of Newcastle disease. For IBD: not fewer than 90% of chickens should survive and show no notable clinical signs of disease nor degree 3 lesions of the bursa of Fabricius.

Three field trials were performed, in one trial challenges with MDV, NDV and IBDV were performed in the laboratory on chickens taken from the field in order to confirm efficacy.

Laboratory trials

The applicant has not discussed the establishment of challenge models. The studies were performed in accordance with the respective Ph. Eur. monographs. This includes a specific (ND) or general (IBD, MD) description of the model. The epidemiological relevance of the challenge strains used for MD- and IBD-challenges was justified. The isolation history of both MDV (RB1B) and IBDV (CS89) challenge strains is described and considered acceptable.

Onset of immunity

Eleven (11) studies were carried out in one day-old chicks in compliance with Ph. Eur. requirements to investigate the onset of protection, by the recommended administration route.

MD

A study for OOI against MD was performed in accordance with Ph. Eur. monograph 0589 requirements. One hundred 1 day-old SPF chicks were vaccinated either by SC injection with Innovax-ND-IBD or received an injection with diluent only. Challenge was performed at 9 days post vaccination by intramuscular injection of a dose of RB1B virus known to induce 95% MD. All chicks were observed for 75 days post challenge for any clinical signs of MD, thereafter all remaining birds were autopsied and macroscopic lesions recorded. The study was valid with respect to the number of animals, the percent of disease in control animals and the percentage of surviving chickens in the period between vaccination and challenge. A below minimum potency dose of vaccine was used ($10^{3.0}$ PFU) at the highest passage number (MSV+6). The calculated relative protection percentage of 87.8% in the vaccinates meets the required minimum of 80% RPP.

In conclusion, the efficacy of Innovax-ND-IBD to reduce mortality, clinical sings and lesions caused by MDV challenge in SPF chicks was considered demonstrated in this study, with an OOI of 9 days.

ND

A study for onset of protection against ND was performed in accordance with Ph. Eur. monograph 0450. Two groups of 24 day-old SPF chicks were vaccinated subcutaneously with Innovax-ND-IBD. Two groups of 12 chicks remained as unvaccinated controls. Animals were challenged either at day 29 or at day 57 using a velogenic NDV strain. Chicks were observed for clinical signs for 15 days after challenge. The study was valid with respect to the number of animals, the percent mortality in control animals and the percentage of surviving chickens between vaccination and challenge. The vaccine used was at minimum potency ($10^{3.3}$ PFU) and maximum passage level. A 100% protection against ND after challenge at 4 weeks meets the required minimum of 90% protection.

In conclusion, the efficacy of Innovax-ND-IBD to reduce mortality and clinical signs caused by NDV is demonstrated in this study, with an OOI of 4 weeks.

IBD

A study for OOI against IBD was performed in accordance with Ph. Eur. 0587 requirements. Two groups of 25 day-old SPF chickens were vaccinated with Innovax-ND-IBD by SC injection and one group of 15 chicks remained unvaccinated. Challenge was performed at two weeks post vaccination using IBDV CS89 challenge virus. Birds were monitored for clinical signs for 10 days post challenge, any surviving animals were necropsied and bursa lesions were scored in accordance with Ph. Eur. 0587. The study was valid with respect to the number of animals, the percent of clinical disease in control animals, the degree of bursal lesions in control animals and the percentage of surviving chickens between vaccination and challenge. Two batches of vaccine were tested in parallel. In one group the requirements for immunogenicity were met in full, with 100% protection from clinical signs, mortality and severe bursal lesions. In the second group clinical signs and mortality were fully prevented, however severe bursal lesions were found in 25% of animals.

Although it is noted that clinical signs and lesions in controls exceeded the minimum requirements of the monograph, it cannot be disregarded that one of two vaccine batches did not meet the requirement for immunogenicity.

OOI against IBD was also investigated in another study. One day-old SPF chicks were used in the study. One group was vaccinated with Innovax-ND-IBD; another group served as non-vaccinated control group. The groups were challenged with IBDV CS89 at 14 or 21 days post vaccination respectively. The study was designed in accordance with Ph. Eur. 0587 requirements. The vaccine used was below the minimum titre and at the maximum passage level to be used in production.

After challenge at two weeks post vaccination, the results did not meet the requirements for immunogenicity. The OOI was however demonstrated at 3 weeks post vaccination, with 100% protection from mortality, clinical signs and bursal lesions.

The applicant provided data on seven (7) additional studies regarding the OOI against IBDV in response to questions:

In the first series of studies, the level of protection was evaluated at different challenge doses. In these studies chickens were vaccinated with a batch at minimum dose and challenges were performed with 15, 7.5, 3.75 and 1.87 CID_{50}/dose of challenge virus, respectively. The 1.87 CID_{50} challenge dose was found to be too low, as none of the controls (or vaccinates) were affected; this challenge is therefore not valid. In the other challenges 91% or more control birds showed clinical signs and 100% of the birds were positive for IBD. However, variable levels of protection were obtained (54.5 – 100% protection) in the vaccinated groups; the vaccinated groups in the lowest and highest challenge dose failing the monograph requirement of ≥ 90% protection and the vaccinated group in the intermediate challenge

passing.

In the second series of studies, groups of chickens were vaccinated at different vaccine doses and challenged with different doses of the challenge virus. In these studies the birds were vaccinated at minimum dose ($10^{3.3}$ PFU) or at slightly higher doses ($10^{3.4}$; $10^{3.5}$ PFU). Challenge was performed at 7.5 CID_{50}, 15 CID_{50} or 30 CID_{50} dose. Challenge was successful in all studies with over 93% of the control chickens showed clinical signs and 100% IBDV positive. In these studies full protection (100%) was seen for 8 out of the 9 vaccinated groups. Only in the study with the highest challenge dose (30 CID_{50}), one group vaccinated slightly above the minimum dose ($10^{3.4}$ PFU/bird) failed the monograph requirement of a 62.5% demonstrated protection.

What can be concluded from the provided data is that the protection conferred by a minimum dose of $10^{3.3}$ PFU of the vaccine at the proposed onset of immunity of 2 weeks is not robust. Although 100% protection is achieved in some experiments (n=5), this is not true for other experiments (n=3: 75%, 65.2%, 54.5%), even at the lowest challenge dose.

The claimed OOI of 3 weeks against IBDV is however sufficiently supported by the data generated in one study (see above), where 100% protection was achieved at 3 weeks post vaccination with a below-minimum dose of $10^{2.9}$ PFU.

Duration of immunity

DOI was addressed separately for the three diseases targeted by the vaccine. Two laboratory studies have been conducted to evaluate the DOI against ND and IBD.

MD

No studies were performed to determine DOI against MD since HVT causes a persistent infection which is considered to provide protection for the entire risk period. The applicant's justification is accepted.

ND

DOI against ND was assessed in the same study as the OOI against ND. At 8 weeks post vaccination, 90.9% of birds were found protected. This level of protection meets the Ph. Eur. monograph 0450 requirement of ≥90% and DOI of 8 weeks is therefore considered demonstrated.

IBD

DOI against IBD was performed in SPF birds, in accordance with Ph. Eur. monograph 0587 requirements. One day-old SPF birds were used in the study. One group was vaccinated with Innovax-ND-IBD; another group served as non-vaccinated control group. The groups were challenged with IBDV CS89 at 56 days post vaccination. The vaccine used was below the minimum titre and at the maximum passage level to be used in production. With 100% protection from mortality, clinical signs and bursal lesions, DOI was demonstrated to be at least 8 weeks.

In conclusion, 8 weeks post vaccination, which is the claimed DOI, significant difference in protection was demonstrated between vaccinated groups and controls, supporting sufficiently the proposed duration of immunity.

Maternal derived antibodies (MDA)

The influence of MDA on the efficacy of the vaccine was studied for each of the three target diseases in three laboratory studies.

MD

Two groups of 50 commercial broiler chicks with confirmed levels of MDA were vaccinated and subsequently challenged with MDV. Chicks were vaccinated at day old with Innovax-ND-IBD or received an injection with diluent only. The study was performed in accordance with Ph. Eur. monograph 0589 requirements for immunogenicity studies. After challenge at day 9, birds were observed for 63 days for clinical signs of MD. The relative protection percentage in MDA+ birds was calculated to be 73.1%. This was found to be a statistically significant effect. No direct comparison of efficacy in MDA+ and MDA- birds can be made from the results, which can be accepted since a challenge was performed. It is however noted that the 73.1% RPP for MDA+ birds is below the 87.8% RPP calculated for the MDA- birds in the OOI study and below the Ph. Eur. monograph requirement of ≥80% RPP. The MDA titres against MDV, NDV and IBDV are reported and shown to be comparable to what is commonly found in the field.

In conclusion, in accordance with the claims, a statistically significant reduction in mortality, clinical signs and lesions caused by MDV was achieved in MDA+ commercial birds.

ND

One-day old commercial broilers were used to study OOI against ND in MDA+ birds. The study was designed in accordance with the Ph. Eur. monograph 0450 test for immunogenicity. Twenty five birds were vaccinated at day-old with Innovax-ND-IBD while 15 birds remained as non-vaccinated controls. Birds were challenged at day 43, a time point when antibodies were deemed sufficiently low. After challenge birds were monitored for clinical signs for 15 days.

The Ph. Eur. validity criteria were not fully met; however this is not a requirement for MDA studies. Significant reduction of mortality and clinical signs, in accordance with the claims, was observed in commercial broiler chicks with confirmed MDA against NDV and MDV, after challenge at 6 weeks post vaccination.

IBD

The study for OOI against IBD in MDA+ birds was performed using commercial broiler chicks and was designed in accordance with the Ph. Eur. monograph 0587 test for immunogenicity. Two groups of 24 day-old chicks were vaccinated with Innovax-ND-IBD. Two groups of 16 and 18 birds were kept as challenge controls, an additional 15 birds were kept as unvaccinated unchallenged controls. One group of vaccinates and one group of controls was challenged at week 5, the two remaining groups were challenged at week 7. After challenge, birds were observed for 10 days for clinical signs of IBD, surviving birds were necropsied and bursal lesions scored. The validity criteria of the relevant Ph. Eur. monograph were not fully met since no clinical signs or mortality were observed in either group. However this is not a requirement for MDA studies and the bursal lesion scores were satisfactory (≥4 in 100%) in controls after challenge at 7 weeks post vaccination. Significant protection (95%) from bursal lesions was observed in commercial broiler chicks with MDA against IBDV, after challenge at 7 weeks post vaccination.

No clinical signs or mortality due to IBDV were observed in the study, however this cannot be expected in 7 weeks old birds. Clinical signs in older animals may however be caused by secondary infections under field (but not experimental) circumstances. The applicant claims the observed prevention of bursal damage will protect older birds from clinical signs and mortality due to secondary infections. This is considered acceptable since enhanced susceptibility to infections is a common consequence of IBDV infection.

From the above it was concluded that vaccination against the three target diseases by the

recommended route with doses of the minimum content recommended in the SPC was efficacious in MDA positive chickens.

Interactions

The interaction with regard to efficacy between Innovax-ND-IBD and ND or IB vaccines of the applicant when applied on the same day but via different administration routes was investigated in seven (7) studies.

With regard to associated use with Nobilis ND Clone 30 or Nobilis ND C2 the following results were obtained.

MD

An OOI study for MD was designed in accordance with Ph. Eur. monograph 0589. Four groups of 60 day-old SPF chicks were used in the study. Group 1 was vaccinated with a standard dose of Innovax-ND-IBD subcutaneously, group 2 was vaccinated with a standard dose of Innovax-ND-IBD SC followed by a standard dose of Nobilis ND C2 by oculonasal administration, group 3 was vaccinated with Innovax-ND-IBD SC followed by a standard dose of Nobilis ND Clone 30 by oculonasal administration, group 4 remained as unvaccinated controls. Challenge was performed at day 9 post vaccination with a high dose of MDV strain RB1B. All birds were monitored for clinical signs until 70 days post challenge and the study was valid with respect to the level of disease in controls and the percentage of surviving animals up to the time of challenge. The requirement of 80% RPP was not fully met for the Innovax-ND-IBD vaccinated group (75%), but was met for both concurrently vaccinated groups (81.8% and 79.6%).

It can be concluded from this study that associated (concurrent) use with Nobilis ND C2 or Nobilis ND Clone 30 does not negatively impact protection of Innovax-ND-IBD against MDV.

ND

An OOI study for ND was designed in accordance with Ph. Eur. monograph 0450 requirements, and was of appropriate quality. Three groups of one day-old SPF chicks were included in the study. Group 1 (n=24) was vaccinated with a standard dose of Innovax-ND-IBD SC, group 2 (n=24) with a standard dose of Innovax-ND-IBD followed by a standard dose of Nobilis ND C2 via the oculonasal route and group 3 (n=12) was left unvaccinated. All animals were challenged at 2 weeks post vaccination with NDV Herts 33/56 and monitored for 14 days for the appearance of clinical signs. Partial protection was obtained in the group vaccinated with Innovax-ND-IBD (21%), which is not unexpected since the claimed OOI against NDV for Innovax-ND-IBD is 4 weeks post vaccination.

However, it can be concluded interference was not observed with the associated use since 100% protection was obtained in the animals vaccinated with Innovax-ND-IBD and Nobilis ND C2, confirming the claimed OOI of 2 weeks post vaccination when Nobilis ND C2 is used concurrently with Innovax-ND-IBD.

The concurrent use with Nobilis ND Clone 30 or Nobilis ND C2 was studied as part of one study. The study was performed in accordance with Ph. Eur. monograph 0450 requirements. Birds were challenged at 3, 4 and 8 weeks post vaccination with virulent NDV. Chickens were monitored for 14 days after challenge for clinical signs specific for NDV infection. In groups vaccinated with Innovax-ND-IBD and Nobilis ND C2, protection was 100% after challenge at 3, 4 or 8 weeks, when Innovax-ND-IBD was combined with Nobilis ND Clone 30 protection was 100%, 95.8% and 100%, respectively.

It can be concluded that OOI and DOI against NDV is not negatively affected by concurrent use of

Innovax-ND-IBD and Nobilis ND C2 or Nobilis ND Clone 30. The claimed OOI against NDV of 3 weeks for concurrent use of Innovax-ND-IBD and Nobilis ND Clone 30 is considered supported.

IBD

Onset of protection against IBD after concurrent use with Nobilis ND Clone 30 was studied as part of a study GNI/0045/16. The study was designed and valid in accordance with Ph. Eur. monograph 0587 requirements. Four groups of 22 day-old SPF chicks were vaccinated with a standard dose of Innovax-ND-IBD, in group 2, 3 and 4 this was followed by vaccination with Nobilis ND Clone 30, Nobilis IB Ma5 or Nobilis IB 4-91, respectively. A fifth group of 14 birds remained unvaccinated. Birds were challenged at two weeks post vaccination. In the control vaccinated group 100% protection was achieved, while in the concurrently vaccinated group 95% protection was found.

OOI and DOI against IBD after concurrent use with Nobilis ND C2 or Nobilis Clone 30 were investigated as part of another study. The study was designed and valid in accordance with Ph. Eur. monograph 0587 requirements. Birds were challenged at 2, 3 and 8 weeks post vaccination. After challenge at two weeks, protection was 56% in the Innovax-ND-IBD + Nobilis ND C2 group and 72% in the Innovax-ND-IBD + Nobilis ND Clone 30 group. After challenge at 3 and 8 weeks, all groups were 100% protected.

The OOI and DOI after concurrent use with these ND vaccines are therefore considered supported by the data.

With regard to associated (concurrent) use with Nobilis IB Ma5 or Nobilis IB 4-91 the following results were obtained.

MD

A study was performed to study concurrent use of Innovax-ND-IBD with both IB vaccines with respect to efficacy against MD. Three groups of 60 day-old SPF birds were used in the study. Group 1 was vaccinated with Innovax-ND-IBD at a standard dose and group 2 was vaccinated with Innovax-ND-IBD followed by oculonasal administration of one dose of Nobilis IB Ma5 mixed with one dose of Nobilis IB 4-91. Group 3 was inoculated with the diluents only. Animals were challenged with RB1B at day 9 post vaccination. Chicks were observed for 70 days post challenge for clinical signs of MD thereafter surviving birds were autopsied and lesions recorded. The study was appropriately designed and executed, in accordance with Ph. Eur. monograph 0589 requirements.

No evidence was obtained for reduction of efficacy against MD due to concurrent administration of IB vaccines. However, the protection obtained against MD by Innovax-ND-IBD (RPP 76.1%) was not sufficient in accordance with Ph. Eur. 0589 (RPP: ≥80%).

In the reply to questions regarding the apparently variable efficacy against MD the applicant has provided an additional study in which the dose of challenge virus was titrated downward.

SPF chicks (n=30) were allocated randomly to one of nine treatment groups. Groups 1, 2 and 3 were vaccinated with a minimum dose (3.3 $\log^{10}$ PFU/dose), groups 4, 5 and 6 with a minimum dose of a different batch while groups 7, 8 and 9 were not vaccinated. At day 9, all birds were challenged with RB1B vvMDV challenge strain IM at dilutions of 1:200 (groups 1, 4 and 7), 1:400 (groups 2, 5 and 8) or 1:800 (groups 3, 6 and 9).

Birds were monitored daily for clinical signs and mortality for 71 days post challenge. The lower challenge dose (1:800) resulted in >80% protection in both vaccinated groups in accordance with Ph.Eur., while the percentage of MD positive birds in the control group was higher than the Ph.Eur. requirement of ⩾70%. In groups challenged with a higher challenge dose, the relative protection percentage did not reach 80% in all groups. When the average (avg) relative percentage protection

(RPP) over the two batches is calculated for each RB1B dilution, it is clear that the RPP increases with decreasing challenge dose (RB1B dilution 1:200=avg RPP: 77.5%, 1:400= avg RPP: 85.4%, 1:800= avg RPP 88.2%).

Thus, it can be concluded that the severe challenge employed in the other studies has likely caused a reduced RPP. It is however agreed that a high level of protection was still achieved in these high challenge groups. And it is also concluded that with a lower challenge dose, that still met the Ph. Eur. requirements in the controls, a RPP of ⩾80% was achieved for two batches of vaccine.

ND

A study was performed to study the efficacy against ND after associated (concurrent) use with the two IB vaccines. Two series of four groups each were used in the study. The first series received a challenge at 4 weeks post vaccination, the second series at 5 weeks, with Herts 33/56. Each series contained one control group (n=14) and three vaccinated groups of 24 day-old SPF chicks. Group 1 (and 5) were vaccinated with Innovax-ND-IBD (standard dose), group 2 (and 6) with Innovax-ND-IBD and subsequently Nobilis IB Ma5 via oculonasal inoculation and group 3 (and 7) with Innovax-ND-IBD and subsequently Nobilis IB 4-91 via oculonasal inoculation. The study was appropriately designed and executed and was valid in accordance with Ph. Eur. 0450 requirements. The level of protection achieved met the Ph. Eur. requirement in each vaccinated group at 4 and 5 weeks post vaccination.

No evidence of interference with protection against ND was found when Innovax-ND-IBD was given simultaneously with Nobilis IB Ma5 or IB 4-91, the OOI for ND at 4 weeks was confirmed.

Immunity against NDV after concurrent use with Nobilis IB Ma5 mixed with Nobilis IB 4-91 was investigated. The study was valid in accordance with Ph. Eur. monograph 0450 requirements and birds were challenged at 3, 4 and 8 weeks post vaccination. Concurrent use of Innovax-ND-IBD with Nobilis IB Ma5 mixed with Nobilis IB 4-91 resulted in 54.2% and 81.8% protection at 3 and 4 weeks post vaccination, respectively. The level of protection at OOI (4 weeks) is thus lower than the Ph. Eur. requirement of 90% and lower than expected for a standard dose of Innovax-ND-IBD (100% in OOI study). The applicant provided the following explanation: since both the live IB vaccines (Nobilis IB Ma5 and Nobilis IB 4-91) and the ND challenge virus replicate in the upper respiratory tract, it is possible that the presence of these viruses in the first weeks post vaccination could enhance the clinical impact of the ND challenge. It must be noted concurrent use with Nobilis IB Ma5 mixed with Nobilis IB 4-91 is not claimed.

In conclusion, concurrent use with Nobilis IB Ma5 or Nobilis IB 4/91 was not found to interfere with protection against ND.

IBD

The OOI of immunity against IBD after concurrent use with Nobilis IB Ma5 and Nobilis IB 4-91 was investigated as part of two studies. Studies were appropriately designed and valid in accordance with Ph. Eur. 0587 requirements.

In one study, Nobilis IB Ma5 and Nobilis IB 4-91 were tested separately for interference with efficacy of Innovax-ND-IBD, high levels of protection (95% and 100%, respectively) were observed after challenge with a dose of 15 CID_{50} IBDV CS89 at two weeks and it was concluded there was no interference.

In a second study both IB vaccines were administered mixed, after vaccination with Innovax-ND-IBD. Although high levels of protection were observed at 3 and 8 weeks post vaccination (100% in all groups), it appeared interference was present when birds were challenged at 2 weeks post vaccination.

This resulted in 65% protection for Innovax-ND-IBD versus 24% protection for Innovax-ND-IBD combined with Nobilis IB Ma5 mixed with Nobilis IB 4-91. However, a high dose challenge (6.19 log10 CID_{50} IBDV CS89) was used which may have overpowered the developing response at 2 weeks post vaccination. It can be accepted that OOI against IBDV is 2 weeks when Innovax-ND-IBD is used concurrently with IB vaccines or live ND vaccines of the applicant.

The OOI and DOI of immunity against infectious bronchitis was evaluated after concurrent use of Innovax-ND-IBD with Nobilis IB Ma5 mixed with Nobilis IB 4-91.

Another study was performed in accordance with Ph. Eur. monograph 0442 requirements. Twelve groups of day-old SPF chicks were used in the study. Groups were vaccinated with Innovax-ND-IBD (SC.) alone or followed by vaccination with Nobilis IB Ma5 mixed with Nobilis IB 4/91 (oculo-nasal). Positive (Nobilis IB Ma5 mixed with Nobilis IB 4-91) and negative (non-vaccinated and non-vaccinated, non-challenged) control groups were included. Challenges were performed at either 3 or 6 weeks post vaccination, with either challenge strain IBV M41 or IBV 4/91. Groups vaccinated with Innovax-ND-IBD and Nobilis IB Ma5 mixed with Nobilis IB 4-91 and challenged with virulent IBV M41 or 4-91 at 3 or 6 weeks post vaccination were protected based on ciliostasis results. Protection was 100% except for the IBV M41 challenge at 6 weeks p.v. when it was 95%. All challenge control groups, including those vaccinated with Innovax-ND-IBD only, showed 100% ciliostasis.

It can be concluded protection against M41 and 4/91 IBV challenge strains (≥95%) was in accordance with Ph. Eur. monograph 0442 requirements and thus there were no compatibility issues.

It can be concluded that no interference occurs when the vaccine is administered on the same day but by different routes with IB (Nobilis IB Ma5 or Nobilis IB 4-91) or ND (Nobilis ND Clone 30 or Nobilis ND C2) vaccines, in SPF chickens. An appropriate statement has been included in the SPC.

Field trials

Three combined safety and efficacy field studies were performed in compliance with GCP standards. Two semi-field studies in the UK in which containment was in place because of the nature of the vaccine (live GMO) and one standard field study in the Netherlands.

In the first and second (semi) field studies, no outbreaks of disease occurred. In order to evaluate vaccine efficacy, field vaccinated chickens from the first study were brought to the laboratory and challenged with vvMDV, NDV and IBDV.

MD: Efficacy against MD was evaluated in a controlled study in two groups of commercial broilers, the first group (n=60) vaccinated with Innovax-ND-IBD and the second group (n=50) injected with diluent only. Challenge was performed at 9 days post vaccination with MDV strain RB1B. The study was valid in accordance with Ph. Eur. requirements. In the vaccinates, 87.3% was protected from MD ($p<0.0001$). The RPP was calculated to be 82%.

The results confirm the efficacy of the vaccine in commercial broilers, against challenge with vvMDV.

ND: Efficacy against ND was evaluated in a controlled study using two groups of 20 vaccinated animals and two groups of 10 non-vaccinated controls. Chickens were challenged at 4 and 6 weeks post vaccination with NDV Herts 33/56. Both challenges were valid, with 90% of controls positive for ND at 4 weeks post vaccination and 100% at 6 weeks post vaccination. After challenge at 4 weeks post vaccination, 60% of vaccinates were protected ($p=0.017$) while at 6 weeks post vaccination. 95% were protected ($p<0.0001$).

The results confirm that significant protection against laboratory challenge with NDV is achieved in

commercial broilers.

IBD: Efficacy against IBD was evaluated in a controlled study by challenge of vaccinated and non-vaccinated control groups at 3, 4, 6 and 8 weeks post vaccination with IBDV CS89. Birds were observed for clinical signs and bursal lesions were evaluated. Few clinical signs were observed after all four challenges, however 92% to 100% of controls was positive based on bursal lesion score. The protection against bursal lesions was significant at all time points ($p<0.0001$) with a protection level between 81% and 95%.

The third field trial, performed in the Netherlands, had a focus on safety with a dose of $10^{4.1}$ PFU used for vaccination. No outbreaks of disease occurred. In conclusion, the data showed that the product is effective for the proposed indication in the field.

Overall conclusion on efficacy

In total 21 laboratory and three field studies were performed to evaluate the efficacy, in accordance with relevant Ph. Eur. monographs. OOI and DOI studies in SPF chickens and the influence of MDA were investigated using vaccine batches at or below the minimum dose to be stated on the label.

Reduction of mortality, clinical signs and lesions caused by MDV with an OOI of 9 days post vaccination was demonstrated.

OOI against ND was demonstrated at 4 weeks post vaccination with reduction of mortality and clinical signs.

OOI against IBD was demonstrated at 3 weeks post vaccination with prevention of mortality, clinical signs and bursal lesions.

No studies were performed to determine DOI against MD since HVT causes a persistent infection which is considered to provide protection for the entire risk period. This is accepted. DOI against ND and IBDV was demonstrated at 8 weeks post vaccination.

The influence of MDA on the efficacy of the vaccine was studied for each of the three target diseases using commercial broiler chicks with confirmed levels of MDA. Protection against MD, ND and IBDV was found to be significant in MDA+ birds.

The interaction with regard to efficacy between Innovax-ND-IBD and ND or IB vaccines of the applicant when applied on the same day but via different administration routes was investigated in several studies. Concurrent use with Nobilis ND C2 or Nobilis ND Clone 30 does not negatively affect the protection against MD. OOI against NDV was demonstrated at 2 weeks after vaccination with Innovax-ND-IBD and Nobilis ND C2 while OOI was demonstrated at 3 weeks after vaccination with Innovax-ND-IBD and Nobilis ND Clone 30. DOI against ND was confirmed at 8 weeks for both combinations. The OOI (3 weeks) and DOI (8 weeks) against IBD were supported by the data presented on concurrent use with Nobilis ND C2 and Nobilis ND Clone 30.

Efficacy against MD, ND, IBD and infectious bronchitis (IB) after concurrent use with Nobilis IB Ma5 or Nobilis IB 4-91 was studied. No evidence of interference with immunity to MD or IB was found. With respect to immunity against NDV and IBDV, concurrent use with Nobilis IB Ma5 or Nobilis IB 4-91 was found not to interfere with OOI or DOI.

Since no disease outbreaks occurred in the two semi-field studies or in the third field (safety) study, the approach to challenge field-vaccinated animals in the laboratory can be accepted. The protection achieved against challenge with MDV, NDV and IBDV in these animals was significant and supports the results of the laboratory efficacy studies.

In conclusion, the claimed efficacy of the vaccine is considered sufficiently supported by the data presented.

Part 5 – Benefit-risk assessment

Introduction

Innovax-ND-IBD is a live recombinant HVT strain containing the F gene from NDV and the VP2 gene form IBDV. The vaccine is intended for protection of chickens from MD, ND and IBD.

The product is intended for use in one-day-old chicks for active immunisation in order to reduce mortality and clinical signs caused by NDV, to prevent mortality and to reduce clinical signs and lesions of IBD and to reduce mortality, clinical signs and lesions caused by MDV. The vaccine is given by SC injection.

The proposed vaccination scheme for is one dose.

The application has been submitted in line with requirements of Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The proposed benefit of Innovax-ND-IBD is its efficacy in active immunisation of one-day-old chicks:

- to reduce mortality and clinical signs caused by Newcastle disease (ND) virus,
- to prevent mortality and to reduce clinical signs and lesions caused by Infectious bursal disease (IBD) virus,
- to reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus.

The benefit was demonstrated in a large number of well-designed laboratory and field studies conducted to an acceptable standard.

OOI was established against NDV infection at 4 weeks post vaccination, against MDV at 9 days post vaccination and against IBDV at 3 weeks post vaccination.

DOI of 8 weeks was established for NDV and IBDV. No data are provided for the DOI against MDV infection and this is acceptable as the HVT virus produces a persistent infection providing a lifelong immunity.

Vaccination against the three target diseases was found to be efficacious in MDA positive chickens.

Additional benefits

Innovax-ND-IBD combines protection against three important poultry diseases. This limits the number of times the animals are required to be handled.

Innovax-ND-IBD reduces the need for live attenuated ND and IBD vaccinations, which can have safety concerns. For IBD, protection is claimed from 3 weeks of age and there is no interference from the presence of MDA which is a problem when live IBD vaccine strains are used.

Innovax-ND-IBD was shown to be efficacious when administered with some other vaccines at the same

day but by different routes in SPF chickens.

Risk assessment

The main potential risks:

Quality:

The formulation and manufacture of Innovax-ND-IBD is well described. The batch-to-batch consistency of batches is satisfyingly demonstrated. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

For the target species:

The product is generally well tolerated in the target animal. No adverse reactions were observed after a tenfold overdose of Innovax-ND-IBD by the SC route. The product is a GMO; the vaccine strain was obtained by insertion of genes into a naturally apathogenic vaccine strain, which is known to be safe for chickens. The biological properties (safety, dissemination, spread) of the original strain were not changed by the genetic modification, reversion to virulence could not be demonstrated. The chance of recombination with other strains or other viruses occurring is considered to be negligible.

For the user:

The CVMP concluded that the user safety for this product is acceptable when used according to the SPC recommendations. Appropriate risk mitigation measures are described in the SPC.

The vaccine is filled in glass ampoules and stored in liquid nitrogen, in exceptional cases ampoules may explode upon heating. Appropriate precautions and warnings for safe handling of the ampoules are included in the SPC.

For the environment:

The vaccine virus is shed with feather dust can remain infectious in the environment for prolonged periods. It was demonstrated that spread to turkeys is possible; spread to chickens was not observed but cannot be excluded. HVT in general can infect avian species only, the related vaccine strain Innovax-ILT was shown to be unable to infect mice.

Like its parent HVT strain, the vaccine strain was shown to be fully non-pathogenic to other avian species (turkey, pheasant, quail, duck), limiting the risk to the environment.

For the consumer:

The withdrawal period is set at zero days.

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform

performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Innovax-ND-IBD is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.